Remarks

Claims 11, 17-18, 22 and 24 are here amended, and claims 1-10, 12, 19-21, 26-27, 50 and 52 have been canceled. Applicants reserve the right to pursue canceled and withdrawn claims in this or future applications.

Claim 11 has been amended to have the limitations of claim 12. Support for the amendment can be found in the original description and claims, as described in detail below. No new matter has been added.

Claims 17 and 22 have been amended to correct the dependency on a currently pending claim.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**".

No new matter has been added. Applicants believe that the claims herein are in condition for allowance, which is respectfully requested.

The subject matter of claim 11 as amended is well supported. As a preliminary matter the application provides examples within the scope of the claims. In particular, the description is replete with instances of assaying expression in a population of muting nucleic acid-treated cells, immediately after the population has been provided with a muting nucleic acid. See for example, p.5, showing steps of transforming animal cells, and then assaying the transformed cells for changes in expression; see also p. 7, lines 26-28 (assay of endogenous mRNA in untransfected or transiently transfected cells). This immediate use of the nucleic acid-treated cells is important, because the method of the present invention pointedly omits Capecchi's stated requirement for at least one and preferably two selecting drugs, as shown below. See also p. 8, lines 13-16, and lines 26-29; p. 9 lines 11-12 and 19-21. See also definitions of "transfection" and "electroporation" on p. 11, lines 17-23, and discussion of the transient condition of a vector on p. 11, lines 10-12. See the transfection procedure referenced on p.16, lines 3-18, and p. 6, line 6, incorporating by reference a standard methods paper on this topic.

Given these examples, it is abundantly clear to a person of ordinary skill in the art that the inhibition of expression in the population by addition of the muting nucleic acid is achieved without selection. (Indeed as discussed below, a person of ordinary skill in the art

would know that selection in animal cells is not even possible with the muting nucleic acid disclosed in the application.)

For this reason, the disclosure of the application in this regard satisfies 35 U.S.C.§112. See for example, *W.L. Gore Assoc. v. Garlock, Inc.*, 220 U.S.P.Q.303, 315, 721 F.2d. 1540, 1556 (Fed.Cir.1983), which states that "[p]atents are written to enable those skilled in the art to practice the invention, not the public." See also, *Atmel Corp. v. Information Storage Devices Inc.*, 53 U.S.P.Q.2d 1225, 1230, 198 F.3d. 1382, which states, "[t]he specification would be of enormous length if one had to literally reinvent and describe the wheel."

It is unnecessary for a patent to point out what is omitted in a procedure. In *S3 Inc. v. Nvidia Corp.*, 59 U.S.P.Q.2d 1745, 2001 WL 876905 at p.5, the court (in a decision dated Aug. 3, 2001) pointed out: "...patent documents need not include subject matter that is known in the field of the invention...patents are written for persons experienced in the field of the invention."

The claims as amended are free of the prior art

Applicants have amended independent claim 11 so that this method is now directed a method for muting expression of an endogenous gene in a cultured population of animal cells. The method comprises the steps of: (a) providing a muting nucleic acid composition having a sequence that is homologous to a sequence in the endogenous gene; and (b) delivering the muting nucleic acid into the population of cells under conditions devoid of a selection for integration of the nucleic acid into a chromosomal site, so that expression of the endogenous gene in the population as a whole is inhibited even though such gene's sequence is not therein disrupted.

The amendments to element (b) of claim 11 distinguish the claim by at least three criteria from the cited reference, Capecchi, M, March 1994 *Scientific American* pp. 52-59.

The prior are requires selection

First, Capecchi's targeted gene replacement describes use of selection for a drug resistant marker because "Regrettably, such targeted replacement occurs only in a small fraction of the treated cells...We must therefore sort through the cells to identify those in which targeting has succeeded...we grow the cells in a medium containing two drugs...[i]nclusion of a "positive" selectable maker promotes survival and growth of cells

that have incorporated the targeting vector...[i]nclusion of the "negative" selectable marker helps to eliminate most of the cells that have incorporated the targeting vector at a random location." (Capecchi , p. 56).

Capecchi in fact devotes almost a page of description to two different selection procedures, that are both necessary in order for him to obtain the rare cell that has a proper replacement sequence inserted into the target gene. Capecchi discusses the rigorous selection system, see p. 55, and why these selection procedures are necessary: "[w]hen all goes well, homologous recombination occurs...To isolate cells carrying the targeted mutation, workers put all the cells into a medium containing selected drugs, here a neomycin analog (G418) and ganciclovir. G418 is lethal to cells unless they carry a funtional *neo*' gene, and so it eliminates cells in which no integration of vector DNA has occurred...Meanwhile ganciclovir kills any cells that harbor a *tk* gene, thereby eliminating cells bearing a randomly integrated vector." [Emphases added.]

In contrast, Applicants' claim 11 is a method that involves delivering the muting nucleic acid under conditions devoid of a selection, and expression of the gene in the population as a whole is inhibited. In fact, in the examples, the muting nucleic acid as shown carries only standard bacterial antibiotic resistance determinants, which are non-functional in animal cells, to which claim 11 is limited. These antibiotic resistance determinants are used as a reporter gene (chloramphenicol acetyltransferase) or to determine the plasmid copy number (ampicillin resistance). See the application, p. 17, lines 13-15, and p. 23, lines 22-24. No genes that confer resistance to any chemical capable of killing animal cells are present on the muting DNA.

The prior art requires insertion of a gene replacement sequence into a target chromosomal gene

A second criterion by which Capecchi's gene replacement differes from claim 11 is that Capecchi requires insertion into a target gene on a chromosome of the cell (Capecchi , p. 54). This is clearly stated in the quotation from Capecchi in the section above addressing selection, and is the reason that his successful cell is so rare.

In contrast, Applicant's claimed invention of muting of an endogenous gene in a population of cells requires that in the population as a whole, there is no integration into a chromosomal site and that the gene's sequence is not disrupted.

The prior art operates only on a rare cell, and not on the population as a whole

Third, Capecchi's successful gene insertion is a rare event, so that "[a]pproximately one in a million treated cells has the desired replacement." (Capecchi p. 57; emphasis added). See quotations from Capecchi, *supra*.

In contrast to the one in a million rare integrant of Capecchi, the claimed method requires the population of recipient cells as a whole.

There is no teaching or suggestion in Capecchi to make any changes in his procedure, to any of these three of his required features of his targeted gene insertion, let alone does he suggest changes to all three. Further, not only is there no suggestion to make these changes, there is no indication that even if any one of these changes were made, that they would produce the successful outcome shown by Applicants.

On the basis of the differences due to any one of these amendments to claim 11, let alone all three, Applicants' claim 11 as amended is neither anticipated by Capecchi according to 35 U.S.C. 102, nor made obvious in view of Capecchi according to 35 U.S.C. 103. Therefore claim 11, and the remaining claims that depend directly or indirectly from claim 11, are neither anticipated nor obvious in view of Capecchi.

Applicants respectfully request that the Examiner withdraw rejections of the claim under 35 U.S.C. 102 and 35 U.S.C. 103.

The written description satisfies 35 U.S.C. 112 first paragraph

The Office Action on p. 2 alleges that while the specification is enabling for a method of producing a knockout mouse comprising embryonic stem cells which have been genetically modified, it does not reasonably provide enablement for a method of producing a knock-out animal of any and all species.

This statement is in error for several reasons. It is not the objective of the invention of claim 11 and its dependent claims to produce knockout animals. Claim 11 as amended is not a description of a method of knocking out a gene function by disrupting the gene sequence, since the amended element (b) specifically requires no selection, no gene disruption, and no insertion into a site on a chromosome.

The Office Action alleges that the specification does not provide working examples that demonstrate muting in vivo. However, the working examples provide a range of muting of gene expression. For example, Example 5 on p. 21 of the application, lines 22-23 states,

"...the level of endogenous procollagen mRNA was surprisingly greatly reduced." Further, p. 22 in the same example, lines 2-3 state, "...transcripts of the endogenous gene, although greatly reduced in amount, were clearly visible." Further, p. 22, lines 28-29 state, "...the level of endogenous collagen mRNA was about 7% that of the control cells." Additional examples of various quantities resulting from muting of endogenous gene expression can be found throughout the specification.

Applicants assert that muting of an endogenous gene in a population of cells, referring to reduction or elimination of gene expression, devoid of selection for a rare event such as gene insertion and gene disruption, has been more than adequately described and exemplified.

Applicants urge the Examiner to withdraw rejection of the claims under 35 U.S.C. 112 first paragraph.

The written description satisfies 35 U.S.C. 112 second paragraph

Claims as amended conform to the requirements of 35 U.S.C. 112 second paragraph.

The term "substantially transient" in claim 26 has been deleted by cancellation of this claim.

The expression, "population of cells" is a term of art known to all of ordinary skill in the art of microbiology and cell biology. In the usage in claim 11, the "population" is particularly suited to describe the response of a large number of cells to the muting nucleic acid, by inhibiting gene expression throughout the culture, in the absence of any selection for a rare cell that has engaged in an insertion event into a site on a chromosome. Applicants assert that this expression properly describes the element of "delivering" the muting nucleic acid so that expression of the endogenous gene in the population is inhibited.

The Applicants further assert that a variety of different clonally pure recipient cell types, including transformed cells, revertants of the transformed cells, and non-transformed or normal cells, are provided. See the Example 7 in the specification, p. 23, lines 17-19, which state, "...expression of pro-α(I) collagen for samples electroporated with pWCT1, followed by cell culture for 24-48 h, was dramatically reduced in all cells electroporated with pWTC1, to a level of less than 10% in Rat-1 and v-*fos* transformed cells, and to about 30% in the revertant cells." It is well known by those of ordinary skill in the art of cell biology that Rat-1 cells are normal fibroblasts, and that v-*fos* cells are oncogenically transformed cells.

For at least these reasons, Applicants assert that claim 11 and remaining claims dependent thereon conform to the requirements of 35 U.S.C. 112 second paragraph.

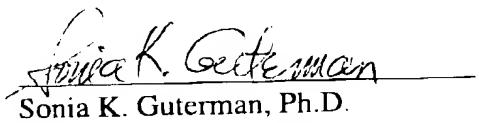
Applicants respectfully request that the rejections of claims on this basis be withdrawn.

Summary

In view of the foregoing amendments and remarks, Applicants submit that the claims are now in condition for allowance. Early and favorable reconsideration of the application is therefore respectfully solicited. The Examiner is invited and encouraged to contact Applicants' representative at the telephone number below if such contact would assist in expediting the present application to allowance.

It is believed that a three month extension of time is required. Applications hereby petition for same and request that the extension fee and any other fee required for the timely consideration of this application be charged to Deposit Account No. 19-4972.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

11. (Twice amended) A method for muting expression of an endogenous gene in a cultured population of animal cells, the method comprising the steps of:

(a) providing a muting nucleic acid composition having a sequence that is homologous to a sequence in the endogenous gene; and

(b) delivering the muting nucleic acid into the population of cells under conditions devoid of a selection for integration of the nucleic acid into a chromosomal site, so that expression of the endogenous gene in the population as a whole is inhibited even though such gene's sequence is not therein disrupted.

17. (Twice amended) A method according to claim [12] 11, wherein the muting transgene sequence is [substantially] homologous to an endogenous sequence [that extends to] comprising a portion of the endogenous gene selected from at least one of the group of: [the] a 5' untranscribed portion, [the] a transcribed coding portion including introns, [the] a 3' untranslated portion, [the] a 3' untranscribed portion, and a portion that overlaps adjacent ends of at least two portion of the endogenous gene.

18. (Amended) A method according to claim 17, wherein the nucleic acid comprises a sequence [that is substantially] homologous to an endogenous sequence located in the 5' portion of the endogenous gene.

22. (Twice amended) A method according to claim [11] 17, wherein the muting nucleic acid comprises a sequence that is [substantially] homologous to an endogenous sequence located at the 3' portion of the gene.

24. (Twice amended) A method according to claim 11, wherein [the step of] delivering the muting nucleic acid in (b) is selected from the group of: transforming, transfecting, electroporating, infecting, and lipofecting the nucleic acid into the cells [at a plasmid copy number which is a multiple of the number of cells to which the nucleic acid is delivered].